



US006118045A

**United States Patent** [19]

Reuser et al.

[11] Patent Number: 6,118,045

[45] Date of Patent: Sep. 12, 2000

[54] **LYSOSOMAL PROTEINS PRODUCED IN THE MILK OF TRANSGENIC ANIMALS**

[75] Inventors: Arnold J. J. Reuser, Rotterdam; Ans T. Van der Ploeg, Poortugaal; Frank R. Pieper, Utrecht; Martin Ph. Verbeet, Amsterdam, all of Netherlands

[73] Assignees: Pharming B.V.; The Universiteit Leiden, both of Leiden; Academic Hospital; Erasmus Universiteit, both of Rotterdam, all of Netherlands

[21] Appl. No.: 08/700,760

[22] Filed: Jul. 29, 1996

**Related U.S. Application Data**

[60] Provisional application No. 60/001,796, Aug. 2, 1995.

[51] Int. Cl.<sup>7</sup> ..... A01K 67/00; A61K 39/395; A61K 35/20; A61K 38/47

[52] U.S. Cl. .... 800/14; 800/7; 800/21; 119/DIG. 1; 424/157.1; 424/535; 424/94.61

[58] Field of Search ..... 119/DIG. 1; 800/2, 800/DIG. 1-4, 14, 7, 21; 435/172.3; 424/450, 157.1, 535, 94.61; 20/23.1; 530/350

[56] **References Cited****U.S. PATENT DOCUMENTS**

5,356,804 10/1994 Desnick et al. .... 435/208  
5,565,362 10/1996 Rosen ..... 435/320.1

**FOREIGN PATENT DOCUMENTS**

WO 93/25567 12/1993 WIPO ..... 800/2

**OTHER PUBLICATIONS**

Belen'kil et al. Purification and Properties of Acid Alpha-Glucosidase (gamma-Amylase) from Human Liver. *Biochemistry*, vol. 40, No. 5, pp. 793-798, 1976.

de Barsey et al. Enzyme Replacement in Pompe Disease: An Attempt with Purified Human Acid Alpha-Glucosidase. *Birth Defects Original Article Series*, vol. 9, No. 2, pp. 184-190, Mar. 1973.

Minamiura et al. Identity of alpha-Glucosidase of Human Kidney with Urine F-1 alpha-Glucosidase. *J. Biochemistry*, vol. 91, pp. 809-816, 1982.

Obara et al. Mutual relationship between milk components and lysosome enzymatic activity in abnormal milk. *Japanese Journal of Veterinary Science*, vol. 45, No. 2, pp. 203-208, 1983.

Oberkotter et al. N-acetyl-beta-hexosaminidase activity in human breast milk. *International Journal of Biochemistry*, vol. 14, No. 2, pp. 151-154, 1982.

Barnes et al. Homology of lysosomal enzymes and related prediction of posttranslational modification sites including phosphorylation of mannose and potential epitopic and substrate binding sites in the alpha and beta subunits of hexosaminidases, 1988.

Bresciani et al. Lysosomal acid phosphatase is not involved in the dephosphorylation of mannose 6-phosphate containing lysosomal proteins. *Eur. J. of Cell Biol.* vol. 58, No. 1, pp. 57-61, Jun. 1992.

Houdebine L. M. Production of pharmaceutical proteins from transgenic animals. *J. of Biotech.* vol. 34, pp. 269-287, 1994.

Agnes G. A. Bijvoet et al., "Expression of cDNA-Encoded Human Acid  $\alpha$ -Glucosidase in milk of Transgenic Mice," *Biochimica and Biophysica Acta*, vol. 1308, 1996, pp. 93-96 (XP000609555).

Henri Rochefort et al., "The Estrogen-Regulated 52K-Cathepsin-D in Breast Cancer: From Biology to Clinical Applications," *Nuclear Medicine and Biology*, vol. 14, No. 4, 1987, pp. 377-384 (XP000609069).

A. T. Van der Ploeg et al., "Intravenous Administration of Phosphorylated Acid  $\alpha$ -Glucosidase Leads to Uptake of Enzyme in Heart and Skeletal Muscle of Mice," *The Journal of Clinical Investigation*, vol. 87, No. 2, Feb. 1991, pp. 513-518 (XP000609448).

Lies H. Hoefsloot et al., "Expression and Routing of Human Lysosomal  $\alpha$ -Glucosidase in Transiently Transfected Mammalian Cells," *Biochemical Journal*, vol. 272, 1990, pp. 485-492 (XP000609457).

Gerard J. Platenburg et al., "Expression of Human Lactoferrin in Milk of Transgenic Mice," *Transgenic Research*, vol. 3, 1994, pp. 99-108, (XP002024692).

Primary Examiner—Deborah J. Clark

Attorney, Agent, or Firm—Townsend & Townsend & Crew

20 Claims, 7 Drawing Sheets

20046130-01602

[57]

ABSTRACT

The invention provides transgenic nonhuman mammals producing phosphorylated lysosomal proteins in their milk, and methods of generating the same. Phosphorylation occurs at the 6' position of a mannose side chain residue. Also provided are methods of purifying lysosomal proteins from milk, and incorporating the proteins into pharmaceutical compositions for use in enzyme replacement therapy.

10046180-011602